PP3A-45
Strategies for membrane protein crystallization

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As part of the Center for Structures of Membrane Proteins (CSMP) our aim is the structure determination of membrane proteins of both bacterial and human origin. Membrane proteins are currently the targets for ~40% of all therapeutic drugs, therefore the structures are expected to provide invaluable information for rational drug design.
Our initial target genes are all 25 *Escherichia coli* membrane kinase receptors, and a handful of human membrane proteins. At least two different constructs are prepared for each target, one as a fusion with Mistic (a novel protein that allows for expression of eukaryotic proteins in *E. coli*), as well as a non-misticated one. We studied the effect of Mistic on protein expression and membrane integration levels of the *E. coli* histidine kinase receptor family. We find that Mistic fusion greatly increases the overall yield, targets the cargo proteins more efficiently to the membrane and may even replace the signal sequence, while the proteins retain kinase activity. The proteins with the highest expression levels have been selected for large-scale preparation and crystallization screening.
Prior to high-throughput crystallization screening a rapid and accurate evaluation of the oligomeric homogeneity of the samples, and the detergent-to-protein ratio in Protein-Detergent Complexes (PDC) is necessary. For this purpose we use an analytical HPLC system in combination with refractive index and static light scattering detectors, and NMR spectroscopy to characterize detergents free in solution and in PDC. Our crystal optimization approaches include automated detergent screening and crystallization under oil. Currently we have obtained crystals for four targets.

PP3A-46
Inhibition studies on mutant Phe91Asn human carbonic anhydrase I (HCA I) gene

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Carbonic anhydrases (CAs; carbonate dehydratases EC 4.2.1.1) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes that and encoded by four evolutionarily different gene families. In mammals, 15 α-CA isozymes or CA-related proteins have been described, with different catalytic activity, subcellular localization and tissue distribution. CAI is a member of α-CA family and situated on the long arm of chromosome 8. It presents in erythrocytes, colon epithelium, lens of eye and corneal epithelium and the most abundant protein after hemoglobin in erythrocytes.
It's known that sulfonamides which are used in glaucoma treatment to reduce inner eye pressure in glaucoma, inhibits hCAI isoenzymes as well as hCA II remarkably. Thus, the aim of our study is to get mutant HCA I enzymes which have low affinity to sulfonamides. In this study hCA I gene was cloned into PGEMT vector from HL60 (Human acute myeloid leukemia cell line) by RT-PCR strategy and subsequently subcloned into pET21a(+) expression vector. Phe91 hydrophobic residue was changed into more hydrophilic Asn residue with PCR based site directed mutagenesis using specific primers. After the expression of wild type and mutant HCA I enzymes in *E. coli* were purified by specific Sepharose 4B-L-Tyrosine affinity gel, hydratase and esterase activities measured. Inhibition manner of these enzymes by Sulphonilamide and acetozolamide widely used for the treatment of glaucoma was investigated.

PP3A-47
New contact site in coiled coil region of human fibrin beta-chain taking part in protofibril lateral association

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Introduction: The transformation of fibrinogen into fibrin by thrombin gives rise to structural rearrangements in the molecule leading to the exposure of the polymerization or contact sites taking part in fibrin intermolecular binding. We obtained monoclonal antibodies (mAbs) to various parts of fibrinogen-fibrin molecule(s) during its transformation into polymeric fibrin and used mAbs as molecular probes to study mechanisms of fibrin polymerization.
Methods: Human fibrin desAABB in 2 M urea was used as an antigen. The epitope for mAb in fibrin molecule was localized using ELISA and immunoblot analysis with various fibrin(ogen) fragments. Turbidity analysis and transmission electron microscopy were used to study the effect of mAbs and their Fab-fragment on fibrin polymerization.
Results: The epitope for mAb FnI-3C proved to be localized in the fibrin fragment Bbeta118-134, which is situated in the coiled coil region of the molecule. MAb FnI-3C and its Fab-fragment inhibited polymerization of fibrins desAA, desAABB and fibrin formed in fibrinogen + -thrombin reaction at equimolar ratio of mAb or Fab to fibrin(ogen). This testifies the blocking of polymerization or contact site(s) by this mAb and its Fab. The turbidity analysis and electron microscopy showed that this mAb and its Fab retarded the stage of fibrin protofibril lateral association. Previously other authors found several amino acid residues localized in coiled coil region of fibrin molecule: BbetaSer111, Bbeta158-160 and BbetaArg166, which participated in fibrin protofibril lateral association.
Conclusion: We suggested that coiled coil fibrin fragment Bbeta118-134 may be fibrin contact site taking part in protofibril lateral binding.

PP3A-48
Design and intracellular delivery of enzymes to enhance metabolic activation of prodrugs and improve anticancer therapy

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Introduction: Nucleoside analogs (NA) are common agents used in chemotherapy of cancer and viral infections. These compounds act by mimicking the physiological substrates of DNA polymerases. They are administered as prodrugs and are then converted to their pharmacologically active, 5'-triphosphorylated states by nucleoside and nucleotide kinases.
Methods: NAs, such as AZT, ganciclovir (GCV), or AraC and gemcitabine, are phosphorylated by different kinases. The rate-limiting reaction is often the first, and in some cases the second phosphorylation step. Through crystal structure analyses of various enzyme-nucleotide complexes, in combination with kinetic measurements, we wish to identifiy variants that are significantly more active and selective than wild-type. Using enzyme-monoclonal antibody-conjugates we target specific cell types.
Results: (1) A mutant of human TMP kinase phosphorylates AZTMP up to 200-fold more efficiently. We evaluated this enzyme/prodrug combination as a novel means to induce cell death in T cells and to kill erythroid leukemia cells in a mouse tumor model. (2) An engineered human deoxycytidine kinase shows improved activation of the prodrugs AraC and gemcitabine. Remarkably, this enzyme is highly active in phosphorylating NAs of the non-physiological stereochemical L-configuration, such as 3TC (lamivudine) and TRO (troxacitabine), that are less toxic *in vivo* and biologically more potent than the corresponding D-enantiomers. Phosphorylation of Ser-74 up-regulates catalytic activity about 10-fold.
Conclusions: Suicide enzyme therapy may become an even more promising strategy to establish control over the fate of cells transduced with integrating viral vectors, and direct protein transduction may lead to the development of novel schemas in nucleoside prodrug-dependent cancer chemotherapy.